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Biological Assay of Lots of Histoplasmin and the Selection of a New Working Lot

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Introduction

Since 1945, an ever-widening interest in the use of histoplasmin has resulted in the publication of many reports on its epidemiological, laboratory and clinical applications, and many other studies are in progress. The most extensive investigations have employed products from the following three sources: (1) Emmons at the National Institutes of Health—product H–3; (2) Christie and Petersen—the "Vanderbilt" product; (3) Howell of the Division of Tuberculosis—lots H–15 and H–40. In addition, the product distributed by Eli Lilly Company is currently in use and may well have extensive use in the future. Other histoplasmins have, of course, been made and used on a limited scale.

Although histoplasmin has been in use for more than 5 years, no standard or reference product exists with which new histoplasmins or the various products previously used can be compared. Valid interpretation of past work and assurance of reasonable comparability and continuity in future work would seem to require adequate comparisons of major lots already used. In addition, they should be compared with lots intended for use in the future. It is unfortunate that relatively little attention has been paid to this important aspect of work with histoplasmin. However, some contributions have been made by Smith (*37*), who compared H–3 and the Vanderbilt histo-

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plasmin, and by workers at the University of Chicago (*58*) who compared the Vanderbilt product with H–15.

The present paper provides some information on the comparability of some of the histoplasmins already extensively used. The principal purpose, however, is to present the assay of a new large batch of histoplasmin which the Division of Tuberculosis proposes to use in the immediate future for its own studies and those of its collaborators. It seems appropriate at this point to review briefly the work of the Division of Tuberculosis with regard to histoplasmin products used.

At the beginning of the work in 1945, a small amount of H–3 was provided by Dr. Emmons of the National Institutes of Health. Since a supply of this product was not available for very large-scale studies, several relatively small lots of histoplasmin were prepared by one of the authors (Howell) and later pooled to form a product designated as H–15. It was expected, when the use of H–15 was adopted in 1946, that enough had been prepared to last a number of years. By 1948, however, the supply had been so depleted that a new product was needed. Accordingly, another relatively small pooled lot, designated H–40, was prepared for interim use until a more adequate supply could be provided. After testing several proposed lots, a new product, designated H–42, has been prepared. It is expected that the amount now available will be sufficient for the next few years.

The introduction of a new lot of a biological product such as histoplasmin immediately raises the question of how it should be assayed. It was finally decided, on the basis of its principal use, to assay the new product by study of cutaneous reactions in human beings, and to attempt to find that dilution of the new product which would be of equal potency to the previously used 1:1000 dilution of H–15. A field program, planned for other purposes, provided an especially favorable opportunity to carry out this procedure in large groups of persons.

Experimental Procedure

For the sake of brevity and clarity in the following presentation, the 1:1000 dilution of H–15 is called "the standard."

The potency of each of several new lots of histoplasmin was assessed by testing three dilutions against the standard 1:1000 dilution of H–15. The dilutions used were chosen on the basis of preliminary trials with the expectation that the weakest dilution would be less potent and the strongest dilution more potent than the standard. Each dilution was compared with the standard by administering the test antigen in one arm, and at the same time, the standard in the other arm of each of a large number of persons.

A measure of the relative potency of each dilution of the test lot was established by utilizing the following two percentages:

1. The percentage of persons who developed smaller reactions to the particular dilution than to the standard;
2. The percentage of persons who developed greater reactions to the particular dilution than to the standard.

If the second percentage is greater than the first, the dilution is considered stronger than the standard; if the opposite occurs, the test dilution is considered weaker. The algebraic difference between these two percentages constitutes a sensitive index of potency and is called the *critical difference*.

If two successive dilutions of the same lot are associated with a reversal of the sign of the critical difference; i. e., from negative to positive, such dilutions bracket the desired matching dilution. This matching dilution is defined as that dilution which would yield a critical difference of zero; i. e., it would give reactions stronger than the standard as frequently as it would give reactions weaker than the standard. It is estimated graphically or by computation from the critical difference obtained at each of the three dilutions of the test product.

Two criteria were used for grading the lots under consideration. The first was one of an a priori biological judgment, namely, that lots requiring lower concentrations (fewer parts per thousand) to match the standard were preferable. The second criterion involved the examination of the frequency with which a matching dilution gave reactions of essentially the same size as the reactions to the standard. It was felt that lots having large values for this frequency represented good matches to the standard. Further, it was believed that a lot could be considered unacceptable if at the matching dilution the frequency of agreement with the standard was exceptionally low. Some notion of an appropriate minimum was obtained by performing trials of the standard against itself.

Test Procedure

The skin tests were performed in the routine manner of Mantoux tuberculin tests. A dose of 0.1 ml. was introduced into the volar surface of the forearm approximately 4 inches from the elbow. The tests were read at 72 hours, and the largest transverse diameter of the induration, or of the erythema in the absence of induration, was recorded in millimeters.

To avoid any bias which would occur if one arm gave stronger reactions than the other, the standard was alternated from right to left arm in succeeding individuals. *The person reading the tests did*

not know in which arm the standard had been given. There was the further advantage that the comparison would not be biased by lighting conditions or other factors which might affect the readings of one arm as compared with the other. Furthermore, the duplicate testing referred to above, in which the standard material was injected in both arms, was interspersed throughout the testing of most of the new lots. Thus the reader did not know which persons had had such identical tests.

Simultaneously with the testing operations, the field staff assessed the clinical accuracy of the work with respect to cooperation of patients and adequacy of technical detail. If any tests were considered unsatisfactory, due record was made of this fact, and the results of such tests have been excluded. Less than 5 percent of nearly 13,000 paired tests were excluded from the analysis.

The comparative tests were performed in the course of tuberculin and histoplasmin surveys conducted in institutions operated by the Department of Welfare of the State of Ohio. For each institution the prevalence of sensitivity to H–15 is indicated in table 1 which also shows the lots tested at each place.

Table 1. *Histoplasmin sensitivity*[1] *and lots tested against H–15 at each institution*

Name of institution	Number tested	Percent reactors[2]	Lots tested against H–15
Columbus State School	1,217	52.2	H–38, H–15.
Columbus State Hospital	1,269	62.1	H–36, H–39, H–15.
Orient State School	1,917	66.0	H–37, H–40, H–15.
Columbus Penitentiary	1,823	70.0	H–41, H–42, H–15.
Dayton State Hospital	990	61.9	H–41, H–42, H–15.
Cleveland State Hospital	1,604	31.4	H–43, H–44.
Cincinnati State Hospital	937	72.0	H–38, H–41, H–42.
Delaware Girls' Industrial School	224	50.9	Lilly (B–8287).
Marysville State Reformatory	310	50.0	H–42.
Mansfield State Reformatory	1,427	53.5	Lilly, H–42, H–15.
Lancaster Boys' Industrial School	601	43.8	H–42.
All institutions	12,319	57.2	

[1] To standard (1:1000 H–15).
[2] Reactor is person responding with 5 mm. or more of induration.

Materials

Each of the several lots of histoplasmin employed in this study was prepared by a method similar to that employed by Emmons et al. (*42*). The medium employed, incubation time, and strain of *Histoplasma capsulatum* for each lot are shown in table 2.

Lot H–15 histoplasmin was prepared in July 1946 by pooling portions of lots H–2, H–4, H–5, H–6, and H–7; lot H–40 was prepared in January 1948 by pooling portions of lots H–9, H–11, and H–13.

Table 2. *Media, incubation periods and strains of* Histoplasma capsulatum *used in the preparation of specified lots of histoplasmin*

Lot number	Date harvested	Medium	Incubation period (days)	Strain
H-2	February 1946	Long's synthetic with 1 percent bactodextrose.	[1] 201 [1] 155	American type culture collection No. 8136.
H-4	do	do	201	See De Monbreun, W. A. (*51*).
H-5	do	do	159	Portuondo, B. C.[2]
H-6	March 1946	B. A. 2[3]	128	See Rhodes, P. H. et al. (*52*).
H-7	do	B. A. 2	[1] 138 [1] 113	Peterson, J. C.[2]
H-9	do	do	[1] 141	See De Monbreun, W. A. (*51*).
H-11	April 1946	do	160	American type culture collection No. 8136.
H-13	do	do	140	See Reid, J. D. et al. (*54*).
H-36	August 1947	do	101	See Rhodes, P. H. et al. (*52*).
H-37	do	do	102	Emmons, C. W.[3]
H-38	do	do	103	Peterson, J. C.[2]
H-39	do	do	105	See De Monbreun, W. A. (*51*).

[1] In part.
[2] Personal communication.
[3] Synthetic medium employed by Emmons, C. W. et al. (*42*).

Results

Tests With Materials of Known Potency

Before the results obtained with the various lots tested are considered, the method of analysis will be illustrated by the consideration of results of tests of serial dilutions of the standard itself. It will be shown that the analytical procedure is quite successful in detecting twofold differences in dilution.

Tests were made with 0.5, 2 and 4 parts per 1,000 of H–15 against the 1:1000 dilution of the same material. Each trial dilution was tested on a separate group of persons. The detailed results for each comparison are presented in correlation tables employing certain arbitrary class intervals (table 3). The number in each cell represents the number of persons with a reaction to the standard dilution of the degree specified at the top of its column, and a reaction to the test dilution of the degree specified at the left side of its row.

Each detailed correlation table can be reduced to the three desired summary figures:

1. The sum of the figures above the diagonal represents the number of persons whose reactions to the test material were smaller than to the standard.

2. The sum of the figures below the diagonal represents the number of persons whose reactions to the test material were greater than to the standard.

3. The sum of the figures on the diagonal represents the number of persons whose reactions to the test material were essentially equal to their reactions to the standard. Persons who showed no reaction to either product are excluded from this sum, because they do not

Table 3. *Correlation between degree of response [1] to specified dilution of lot H–15 and to the standard 1:1000 dilution of the same product*

0.5:1000 H–15 × 1:1000 H–15

	A	B	C	D	E	F	G	H	Total
A	31	--	1	2	1	1	--	--	36
B	--	--	--	--	--	--	--	--	--
C	1	--	3	3	--	3	--	--	10
D	--	--	--	8	14	6	1	--	29
E	1	--	5	7	14	18	4	--	49
F	--	--	--	4	7	3	1	1	16
G	--	--	--	--	3	2	5	1	11
H	--	--	--	--	--	1	2	--	3
Total	33	--	9	24	39	34	13	2	154

2:1000 H–15 × 1:1000 H–15

	A	B	C	D	E	F	G	H	Total
A	67	--	--	--	--	--	--	--	67
B	--	1	--	--	--	--	--	--	1
C	2	2	1	1	--	--	--	--	6
D	--	--	1	1	--	--	--	--	2
E	--	--	4	2	5	7	--	--	18
F	2	--	--	3	17	26	5	--	53
G	--	--	--	--	2	15	9	2	28
H	--	--	--	--	--	5	4	3	12
Total	71	3	6	7	24	53	18	5	187

4:1000 H–15 × 1:1000 H–15

	A	B	C	D	E	F	G	H	Total
A	51	1	--	--	--	--	--	--	52
B	2	--	--	1	--	--	1	--	4
C	--	1	--	--	--	--	--	--	1
D	4	1	--	4	2	1	--	--	12
E	3	--	2	5	9	3	--	--	22
F	1	--	--	11	11	4	--	1	28
G	--	1	1	4	21	12	3	2	44
H	--	--	--	1	4	7	4	--	16
Total	61	4	3	26	47	27	8	3	179

[1] A = No response.
B = Erythema only, less than 10 mm.
C = Either: erythema only, 10 mm. or more
or: induration of 1–4 mm.
D = Induration of 5–7 mm.
E = Induration of 8–9 mm.
F = Induration of 10–11 mm.
G = Induration of 12–14 mm.
H = Induration of 15 mm. or more.

Table entries represent number of persons.

contribute information about the potency of the two products. Their inclusion would make the degree of agreement dependent on the level of sensitivity in the group used for the tests.

For each of the three dilutions, these summary figures are presented in table 4. The numbers have been converted to percentages, and, for convenience in further discussion, the percentages which yield the critical difference will be called critical percentages.

Table 4. *Comparison of materials of known potency: tests of three dilutions of H–15 against the standard 1:1000 dilution of H–15* [1]

Response	Persons					
	0.5:1000 H–15		2:1000 H–15		4:1000 H–15	
	Number	Percent	Number	Percent	Number	Percent
Total tested	154	----------	187	----------	179	----------
No response to either dilution	31	----------	67	----------	51	----------
Some response to one or both dilutions	123	100.0	120	100.0	128	100.0
Reaction to the test dilution was:						
Smaller than to 1:1000 H–15	57	46.4	15	12.5	12	9.4
Greater than to 1:1000 H–15	33	26.8	59	49.2	96	75.0
Equal to 1:1000 H–15	33	26.8	46	38.3	20	15.6
Critical difference (greater-smaller)	----------	−19.6	----------	36.7	----------	65.6

[1] See table 3 for details. See appendix for details of tables 5–12.

In the group that received the 0.5:1000 dilution, 46.4 percent gave weaker reactions to this dilution than to the standard, while 26.8 percent gave stronger reactions. The negative critical difference, −19.6 percent, indicates that the 0.5:1000 dilution is weaker than the standard. With the 2:1000 dilution the relationship to the standard is completely inverted; the critical percentages are 12.5 percent and 49.2 percent, yielding a critical difference of 36.7 percent. With 4:1000 the difference in potency is further emphasized by a critical difference of 65.6 percent.

As previously stated, the values of the critical differences not only reveal the relative potencies of each particular dilution, but they may be used to provide estimates of the best dilution for matching the standard. Such an estimate can be made graphically as in figure 1. Here the critical differences for each dilution have been plotted against the logarithms of the dilutions.

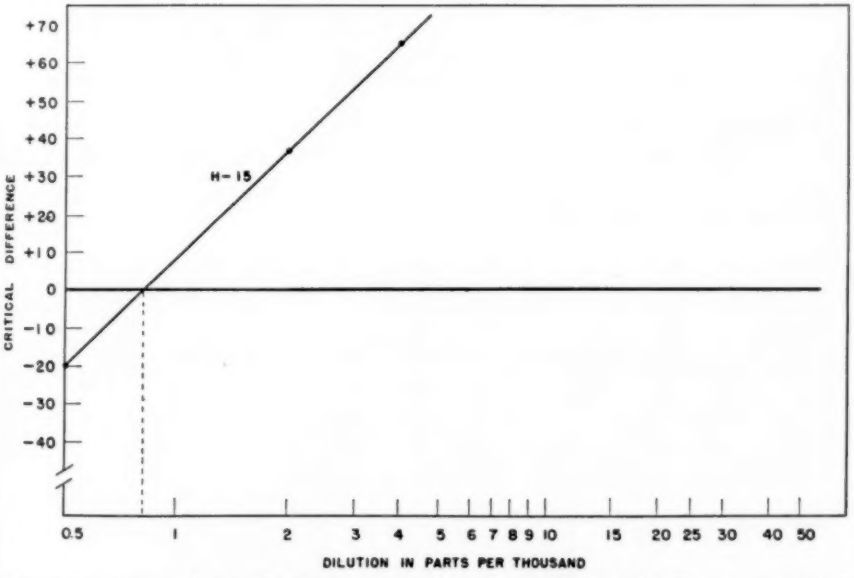

Figure 1. Assay of lot H–15 against itself. The indicated dilutions were tested against the standard 1:1000 preparation.

The relationship between the logarithm of the dilution and the critical difference seems to be linear over the range of dilutions employed in this set of comparisons. In fact, a straight line fits the points exceptionally well. The intersection of this line with the horizontal zero line provides the experimental estimate of the matching dilution, namely 0.8:1000. The deviation of this estimate from the known true matching dilution of 1:1000 is a function of both the statistical reliability of the critical percentages and the basic accuracy of the preparation of the dilutions. Although both of the weaker

trial dilutions were prepared serially from the strongest (4:1000), the three bottles of standard dilution (1:1000) were individually prepared independently of the trial dilutions. These standard dilutions were prepared in the usual manner by adding 0.1 ml. of stock material to 100 cc. of buffer. It can be seen that a small error of 0.01 ml. would mean that the standard material was 0.9:1000 rather than the 1:1000 intended.

These remarks on the accuracy of the comparisons are made to indicate that, although twofold differences in dilutions can be readily detected, it is not claimed that the assay will estimate a matching dilution closer than within 25 percent of the true value.

Table 5. *Comparison of three dilutions of each of four lots of histoplasmin with 1:1000 H–15*

Response	Persons					
	Number	Percent	Number	Percent	Number	Percent
	1:1000 H–36		**3:1000 H–36**		**10:1000 H–36**	
Total tested	209		178		183	
No response to either product	35		45		38	
Some response to one or both products	174	100.0	133	100.0	145	100.0
Reaction to the test product was—						
Smaller than to 1:1000 H–15	151	86.8	63	47.4	32	22.1
Greater than to 1:1000 H–15	12	6.9	26	19.5	68	46.9
Equal to 1:1000 H–15	11	6.3	44	33.1	45	31.0
Critical difference (greater-smaller)		−79.9		−27.9		24.8
	1:1000 H–37		**5:1000 H–37**		**20:1000 H–37**	
Total tested	258		162		208	
No response to either product	41		53		61	
Some response to one or both products	217	100.0	109	100.0	147	100.0
Reaction to the test product was—						
Smaller than to 1:1000 H–15	208	95.9	83	76.2	63	42.8
Greater than to 1:1000 H–15	5	2.3	7	6.4	32	21.8
Equal to 1:1000 H–15	4	1.8	19	17.4	52	35.4
Critical difference (greater-smaller)		−93.6		−69.8		−21.0
	1:1000 H–38		**3:1000 H–38**		**10:1000 H–38**	
Total tested	386		275		425	
No response to either product	163		138		156	
Some response to one or both products	223	100.0	137	100.0	269	100.0
Reaction to the test product was—						
Smaller than to 1:1000 H–15	187	83.9	90	65.7	106	39.4
Greater than to 1:1000 H–15	13	5.8	11	8.0	48	17.8
Equal to 1:1000 H–15	23	10.3	36	26.3	115	42.8
Critical difference (greater-smaller)		−78.1		−57.7		−21.6
	1:1000 H–39		**5:1000 H–39**		**20:1000 H–39**	
Total tested	120		173		168	
No response to either product	29		44		28	
Some response to one or both products	91	100.0	129	100.0	140	100.0
Reaction to the test product was—						
Smaller than to 1:1000 H–15	65	71.4	50	38.8	18	12.9
Greater than to 1:1000 H–15	11	12.1	27	20.9	85	60.7
Equal to 1:1000 H–15	15	16.5	52	40.3	37	26.4
Critical difference (greater-smaller)		−59.3		−17.9		47.8

Assay of Each of Four Lots of Histoplasmin

Four large histoplasmin lots, identified as H–36, H–37, H–38, and H–39, were available. Preliminary trials indicated that they were all weaker than H–15, with H–37 and H–39 appearing especially weak. This information guided the selection of the trial dilutions shown in the summary table of results from tests with each of the four lots (table 5). The detailed correlation tables for each dilution of each lot are provided in the appendix. The critical difference for each lot at each dilution is plotted on figure 2. First approximations to the matching dilution for each lot are obtained by connecting the two points closest to the zero axis.

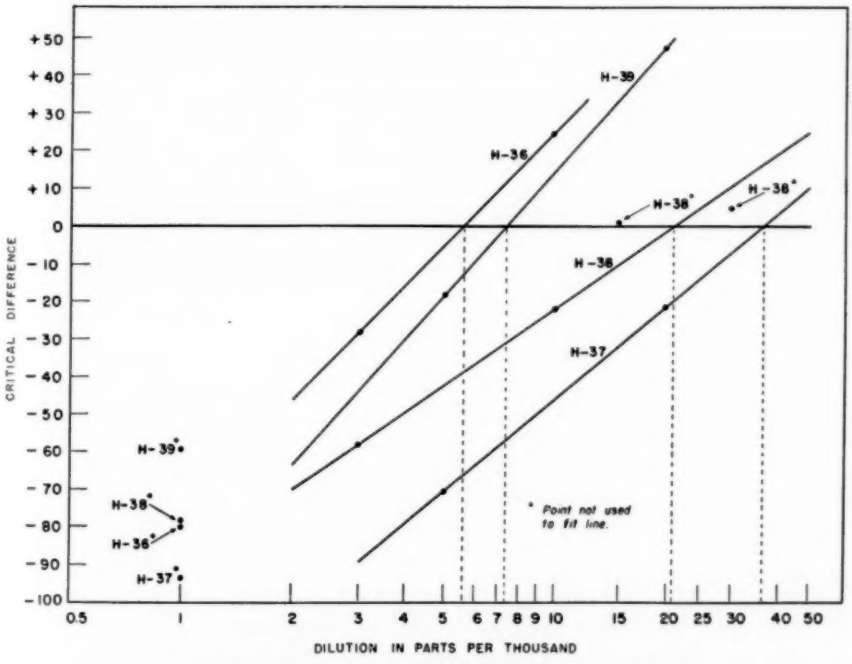

Figure 2. **Assay of lots H–36, H–37, H–38, and H–39 against H–15. The indicated dilutions of each lot were tested against the standard 1:1000 dilution of H–15.**

It appeared appropriate to reject lot H–37 from further consideration because of the high concentration required to match the standard, approximately 35 parts per 1000. H–38 might also have been rejected, but judgment was reserved pending further examination of the data and additional clinical testing.

The data were reviewed to see whether there was any marked variation in the degree of agreement with the standard at the matching dilution of each lot. For this purpose the diagonal sum; i. e., the percentage of persons with the same reaction to the two products, was examined. It was possible, of course, to do this only for the dilutions

tested and not for the matching dilutions. It may reasonably be inferred, however, that the degree of agreement at the matching dilution would not be less than at any other dilution. Therefore, crude estimates were made from the values observed with the dilutions closest to matching. There was no great variation in the degree of agreement among the four lots, which in each case was very satisfactory when compared with the best that might be expected on the basis of the degree to which H–15 agreed with itself. (Data on the self-matching characteristics of H–15 are presented in a later section.) By this criterion H–38 was one of the better lots.

Subsequent tests of two additional dilutions of H–38, 15 and 30 parts per thousand, were performed. The results are shown in table 6 and the two critical differences are also plotted on figure 2. A straight line fitted (not shown) to the three points near the zero axis yielded an estimate of 24 parts per thousand for the matching dilution.

Table 6. ***Comparison of two additional dilutions of H–38 with 1:1000 H–15***

Response	Persons			
	15:1000 H–38		**30:1000 H–38**	
	Number	Percent	Number	Percent
Total tested	90	----------	289	----------
No response to either product	22	----------	48	----------
Some response to one or both products	68	100.0	241	100.0
Reaction to the test product was—				
Smaller than to 1:1000 H–15	19	28.0	53	22.0
Greater than to 1:1000 H–15	20	29.4	63	26.1
Equal to 1:1000 H–15	29	42.6	125	51.9
Critical difference (greater-smaller)	----------	1.4	----------	4.1

Assay of Four Pooled Lots

Since no individual lot appeared to warrant selection it was decided to form a pooled lot. To determine whether the inclusion of H–38 was advisable, pools including and excluding this lot were necessary. Although pooling in equal parts was the natural approach, it was apparent that pooling in proportion to the available supply of each lot would provide a larger volume of the final product.

The requirements of including and excluding H–38 from the pool and of using equal and proportionate volumes of the constituents resulted in the preparation of four separate specimen pools for comparative evaluation. Their designations and composition were:

	H–41	*H–42*	*H–43*	*H–44*
H–36	1	2	1	2
H–38	1	2	--	--
H–39	1	1	1	1

The extensive information on the component lots permitted the selection of trial dilutions for the pooled lots in a narrow range. The results of comparing each of the four pooled lots against 1:1000 H–15 are shown in table 7 and figures 3, 4, and 5. (Detailed tables are in the appendix.)

The tests with both H–41 and H–42 yielded 8:1000 as the estimate for the matching dilution. The degrees of agreement with the stand-

Table 7. *Comparison of specified dilutions of each of four lots of histoplasmin with 1:1000 H–15*

Response	Persons							
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
	4:1000 H–41		**8:1000 H–41**		**8:1000 H–41**		**16:1000 H–41**	
Total tested	418		419		268		188	
No response to either product	114		115		79		42	
Some response to one or both products	304	100.0	304	100.0	189	100.0	146	100.0
Reaction to the test product was—								
Smaller than to 1:1000 H–15	152	50.0	102	33.6	72	38.1	24	16.4
Greater than to 1:1000 H–15	55	18.1	102	33.6	55	29.1	76	52.1
Equal to 1:1000 H–15	97	31.9	100	32.8	62	32.8	46	31.5
Critical difference (greater-smaller)		−31.9		0		−9.0		35.7
	4:1000 H–42		**8:1000 H–42**		**8:1000 H–42**		**16:1000 H–42**	
Total tested	428		254		450		251	
No response to either product	131		69		116		50	
Some response to one or both products	297	100.0	185	100.0	334	100.0	201	100.0
Reaction to the test product was—								
Smaller than to 1:1000 H–15	138	46.5	80	43.2	82	24.6	35	17.4
Greater than to 1:1000 H–15	40	13.5	48	26.0	77	23.0	119	59.2
Equal to 1:1000 H–15	119	40.0	57	30.8	175	52.4	47	23.4
Critical difference (greater-smaller)		−33.0		−17.2		−1.6		41.8
	4:1000 H–43		**8:1000 H–43**					
Total tested	224		530					
No response to either product	140		325					
Some response to one or both products	84	100.0	205	100.0				
Reaction to the test product was—								
Smaller than to 1:1000 H–15	53	63.1	67	32.7				
Greater than 1:1000 H–15	12	14.3	62	30.2				
Equal to 1:1000 H–15	19	22.6	76	37.1				
Critical difference (greater-smaller)		−48.8		−2.5				
	4:1000 H–44		**6:1000 H–44**		**8:1000 H–44**			
Total tested	277		278		295			
No response to either product	172		204		186			
Some response to one or both products	105	100.0	74	100.0	109	100.0		
Reaction to the test product was—								
Smaller than to 1:1000 H–15	56	53.3	23	31.1	17	15.6		
Greater than to 1:1000 H–15	19	18.1	27	36.5	44	40.4		
Equal to 1:1000 H–15	30	28.6	24	32.4	48	44.0		
Critical difference (greater-smaller)		−35.2		5.4		24.8		

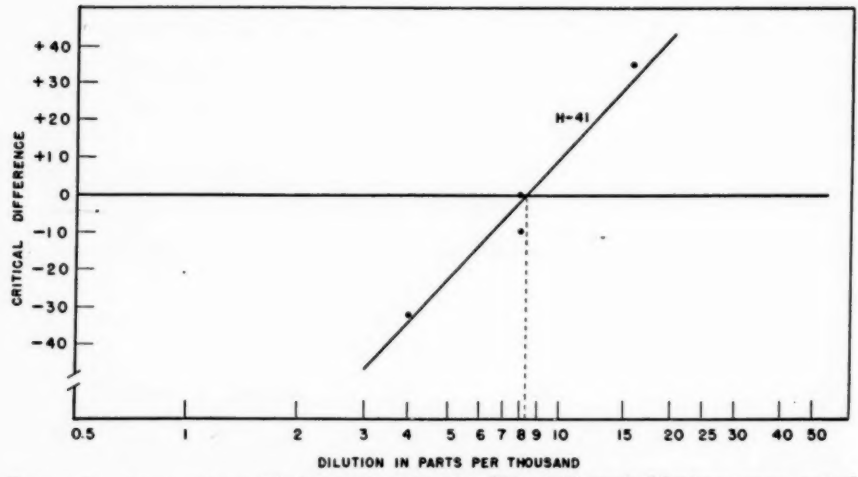

Figure 3. Assay of lot H–41 against H–15. The indicated dilutions were tested against the standard 1:1000 dilution of H–15.

ard at the matching dilution were 33 percent in both trials with H–41 and 52 percent and 31 percent for the two trials with H–42. Thus the evidence somewhat favored H–42 which, in view of its larger size, would have been chosen unless the data had been clearly in favor of H–41.

The tests with H–43, the pool of equal parts of H–36 and H–39 also indicated a matching dilution of 8:1000 (a smaller figure was expected). The agreement with the standard at this dilution was 37 percent. The matching dilution for H–44, the other lot not involving H–38, was 6:1000, and the degree of agreement was 32 percent. Thus there was no apparent superiority of H–43 or H–44 over H–42, and

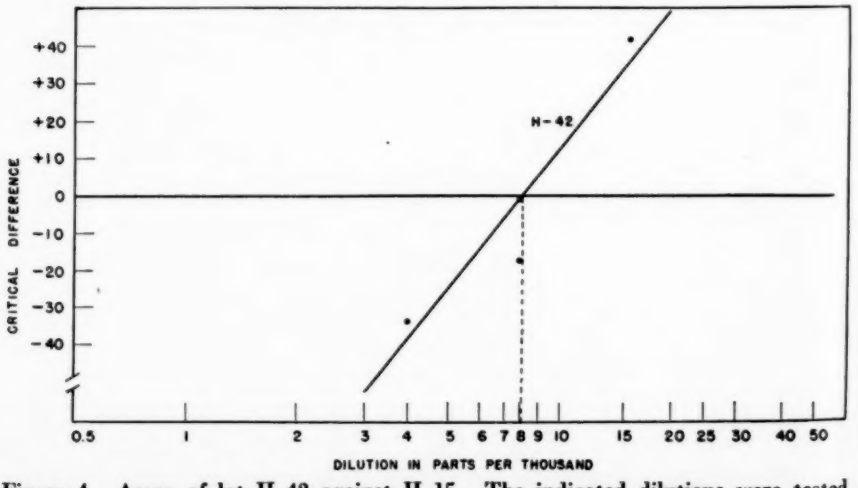

Figure 4. Assay of lot H–42 against H–15. The indicated dilutions were tested against the standard 1:1000 dilution of H–15.

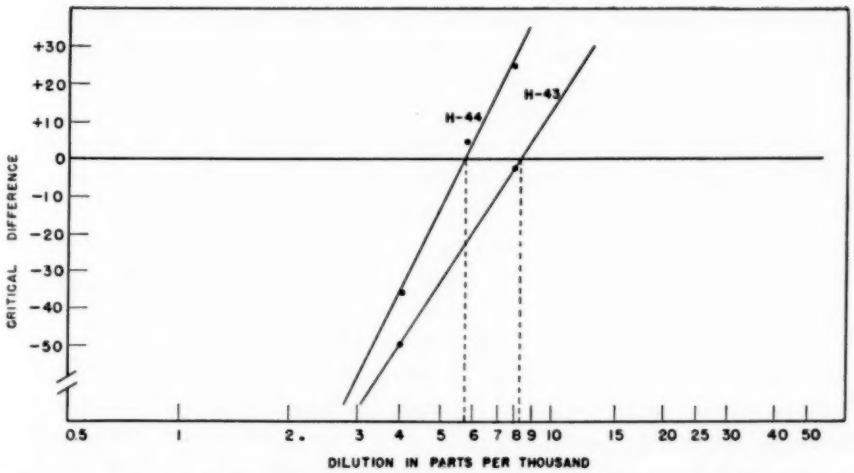

Figure 5. Assay of lots H–43 and H–44 against H–15. The indicated dilutions of each lot were tested against the standard 1:1000 dilution of H–15.

an 8:1000 dilution of H–42 was tentatively selected as the new working lot of histoplasmin for the research activities of the Division of Tuberculosis.

Since the estimated matching dilution of H–42, 8:1000, was close to the more easily prepared 1:100 dilution (10:1000), it was believed of interest to try out this dilution to see if it gave results differing in any great extent from the 8:1000 dilution.

In the first trial, group 1 of table 8. an unexpected result occurred. The critical difference was significantly negative, −29.9 indicating that the dilution was weaker than the standard. Examination of records and procedures did not explain this result. There was, however, an indication that something irregular had happened, since similar tests on 10:1000 H–41 performed at the same institution showed this dilution of H–41 to be weaker than the standard, a

Table 8. *Comparisons of 10:1000 dilution of H–42 with 1:1000 H–15 in 4 groups of persons*

Response	Persons							
	Group I		Group II		Group III		Group IV	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Total tested	272	------	310	------	321	------	601	------
No response to either product	58	------	141	------	132	------	327	------
Some response to one or both products	214	100.0	169	100.0	189	100.0	274	100.0
Reaction to the test product was:								
Smaller than to 1:1000 H–15	102	47.7	42	24.9	46	24.3	22	8.0
Greater than to 1:100 H–15	38	17.8	57	33.7	61	32.3	174	63.5
Equal to 1:100 H–15	74	34.5	70	41.4	82	43.4	78	28.5
Critical difference (greater-smaller)	------	−29.9	------	8.8	------	8.0	------	55.5

result also contrary to expectation. It was concluded that some error had occurred and further tests with 10:1000 H–42 were warranted.

These were conducted in the fall of 1949 at three different institutions. At the first two, the results were consistent with expectation. The critical differences were moderately positive, 8.8 percent and 8.0 percent. At the third, the results were again inconsistent with past experience. The critical difference was 55.5 percent. Review of the detailed correlation table revealed that the differences between the reactions to the two products tended to be quite small. It is probably relevant that this was the only place where the standard procedure of alternating the placement of the standard product in the right and left arms was not followed. It was concluded that the large critical difference resulted from a small error in preparation of materials augmented by a reading bias.

It appeared then that the 1:100 dilution of H–42 was a very close match to the former standard, 1:1000 H–15. It was decided, nevertheless, to adopt 8:1000 H–42 as the new standard, and to provide the convenience of the 1:100 dilution procedure by preparing batches of H–42 so that a 1:100 dilution of such stock would yield the 8:1000 dilution used in these studies. This is achieved by adding 25 percent buffer solution to the pooled material when it is prepared.

Duplicate Tests With 1:1000 H–15

As indicated previously, duplicate tests of the standard were made; i. e., 1:1000 H–15 was injected in both arms of a number of persons at several institutions. These tests provided an estimate of the degree to which a new product might be expected to match the standard, by showing how well the standard could match itself. Each of the comparisons represents duplicate tests from the same bottle of diluted antigen. These duplicate tests also provided extensive information on the relative response of right and left arms.

The results of three sets of duplicate tests, shown in table 9, indicate that the standard agreed with itself, within the limits of the class intervals used, in 42.9 percent, 33.2 percent, and 32.2 percent, respectively, of the reactions observed. As previously indicated these values are not higher than those obtained with the new products.

The comparison of right arm with left arm indicated a very slight predominance of reactions in the left arm. The critical differences for left arm against right arm were 7.7 percent, 2.6 percent, and 7.0 percent, respectively, in the three trials. Additional tests of this kind with H–41, H–42, and 0.0002 mg. PPD indicated that the opposite occurred frequently. Thus it appeared that responses in the two arms could be considered to be essentially the same in the long run.

To ascertain whether different stock bottles of H–15 might have

Table 9. *Comparison of the relative responses in the left and right arms in duplicate tests with 1:1000 H–15*

Response	Persons					
	Group I		Group II		Group III	
	Number	Percent	Number	Percent	Number	Percent
Total tested	268		238		331	
No response in either arm	86		45		104	
Some response in either or both arms	182	100.0	193	100.0	227	100.0
Left smaller than right	45	24.7	62	32.1	69	30.4
Left greater than right	59	32.4	67	34.7	85	37.4
Left equal to right	78	42.9	64	33.2	73	32.2
Critical difference (greater-smaller)		7.7		2.6		7.0

contributed to the erratic results observed in the trials with 10:1000 H–42, a direct comparison of two bottles of stock was made. This comparison also provided an estimate for the broader variation that might be expected in the preparation of supposedly identical products, in that bottle differences and variation in the preparation of dilutions would have a chance to exhibit themselves. Table 10 indicates that there was no essential difference between the bottles, for the critical difference was only −2.2 percent. The degree of agreement between the stock bottles, 36.4 percent, was just as good as that obtained with duplicate tests from the same bottle of diluted antigen.

Table 10. *Comparison of the relative potency of two stock bottles of H–15, diluted 1:1000*

Response	Persons	
	Number	Percent
Total tested	405	
No response to either preparation	177	
Some response to one or both preparations	228	100.0
Reaction to the preparation from bottle No. 2 was—		
Smaller than to bottle No. 1	75	32.9
Greater than to bottle No. 1	70	30.7
Equal to bottle No. 1	83	36.4
Critical difference (greater-smaller)		−2.2

Assay of Lot H–40

Since considerable quantities of lot H–40 have been used pending the selection of H–42, it is appropriate to report the results of tests with this antigen. Preliminary trials indicated that H–40 differed less in potency from H–15 than did the other lots. Therefore trial dilutions closer to 1:1000 were selected. Although three dilutions were employed, most of the comparative tests with one of them, 2:1000, were declared unreliable by the field staff, and therefore the entire set has been excluded from consideration in this assay. The results of tests with the other two dilutions are shown in table 11 and figure 6. The observed critical differences lead to an estimate of

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Table 11. *Comparison of two dilutions of H–40 with 1:1000 H–15*

Response	Persons			
	1:1000 H–40		5:1000 H–40	
	Number	Percent	Number	Percent
Total tested	206		232	
No response to either product	70		48	
Some response to one or both products	136	100.0	184	100.0
Reaction to the test product was—				
Smaller than to 1:1000 H–15	83	61.0	35	19.0
Greater than to 1:1000 H–15	17	12.5	80	43.5
Equal to 1:1000 H–15	36	26.5	69	37.5
Critical difference (greater-smaller)		−48.5		24.5

3:1000 for the matching dilution. Before the refined technique employing critical differences to find the matching dilution was developed, tentative selection of a 2:1000 dilution was made. This dilution has been employed in a number of studies as well as the 1:1000 dilution.

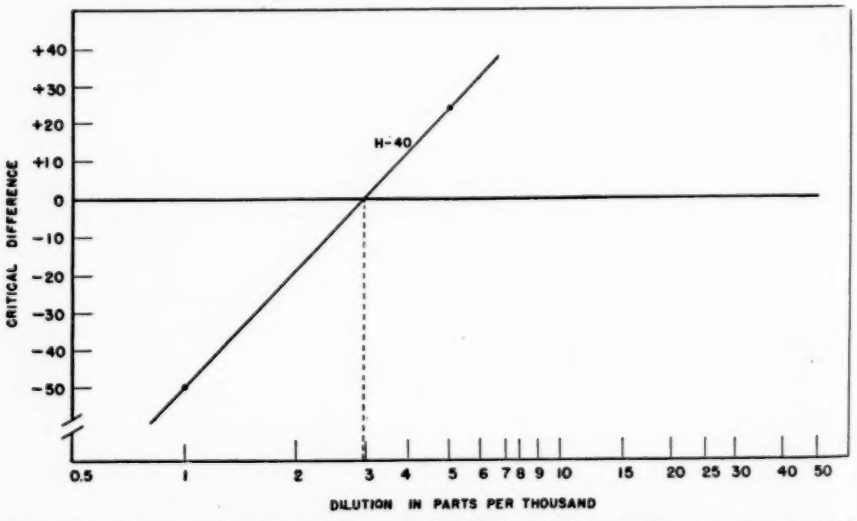

Figure 6. Assay of lot H–40 against H–15. The indicated dilutions were tested against the standard 1:1000 dilution of H–15.

In order to interpret the results of research programs in which H–40 was used, it is desirable to know how much weaker the dilutions employed were than the standard in terms of frequency of reactors. Direct answer to such inquiry may be obtained for the 1:1000 dilution by reference to the detailed results in the appendix. Unfortunately, no data can be presented for the 2:1000 dilution. From the appendix table for 1:1000 H–40 the following summary table may be constructed:

		1:1000 H–15		
		Nonreactors	*Reactors* [1]	*Total*
1:1000 H–40	Nonreactors	72	9	81
	Reactors [1]	1	124	125
	Total	73	133	206

[1] Persons with reactions of 5 mm. or more of induration.

From this table it may be seen that nine of the reactors to H–15 were nonreactors to H–40 while the opposite condition occurred only once. In terms of prevalence of sensitivity, 60.7 percent (125/206) were reactors to H–40, while H–15 elicited reactions in 64.6 percent (133/206). To summarize, 1:1000 H–40 produced 94 percent (125/133) as many reactors as did 1:1000 H–15 in the same group of 206 persons.

The comparisons are similar when a more liberal definition of a reactor is used, namely, a person with any induration, or erythema only of 10 or more mm. Specifically, 1:1000 H–40 was 96 percent efficient, detecting 128 reactors against 133 from 1:1000 H–15. On the other hand, it is interesting to observe that the difference in potency is more clearly manifested when the frequencies of large reactions are compared. For example, 1:1000 H–40 elicited only 9 reactions of 12 mm. or more of induration, while 1:1000 H–15 elicited 24.

Potency of Lilly Histoplasmin

As Smith has stated (*37*) there are now two major sources of histoplasmin, the Division of Tuberculosis and the Eli Lilly Company. A comparison of the Lilly product with H–15 was included in the course of this investigation.

Through the courtesy of Dr. C. G. Culbertson of the Lilly Laboratories, we received a small quantity of Lilly lot B–8287. There are two sets of directions accompanying this material, one for preparing a 1:100 dilution and another for 1:1000, with no indication of the dilution of choice. The results of testing both are shown in table 12,

Table 12. ***Comparison of two dilutions of Lilly Lot B–8287 with 1:1000 H–15***

Response	Persons			
	1:1000 Lilly		1:100 Lilly	
	Number	Percent	Number	Percent
Total tested	224		701	
No response to either product	106		332	
Some response to one or both products	118	100.0	369	100.0
Reaction to the test product was—				
Smaller than to 1:1000 H–15	108	91.5	108	29.3
Greater than to 1:1000 H–15	4	3.4	86	23.3
Equal to 1:1000 H–15	6	5.1	175	47.4
Critical difference (greater–smaller)		−88.1		−6.0

with details in the appendix. The 1:1000 dilution of the Lilly material was much weaker than 1:1000 H–15, yielding a critical difference of −88.1 percent, whereas the 1:100 dilution of the Lilly product with a critical difference of −6.0 percent was just about equal to 1:1000 H–15.

Agreement on Selection of Reactors

It is of interest to examine the extent of agreement of the matching dilution with the standard in number and identity of reactors. For illustration this agreement for H–42 and for the Lilly product is shown in the following extract from the detailed correlation tables.

		1:1000 H–15	
		Nonreactors	*Reactors* [1]
8:1000 H–42	Nonreactors	208	21
	Reactors [1]	14	461
1:100 Lilly B–8287	Nonreactors	340	5
	Reactors [1]	3	353

[1] Reactors: Persons with induration of 5 mm. or more.

In the first group it may be seen that H–15 indicated 482 reactors (68.5 percent) while H–42 indicated 475 reactors (67.5 percent) in the same group of 704 persons. Thus the agreement on prevalence of reactors is excellent. Moreover, there was fairly good agreement between H–15 and H–42 on identity of reactors. The two products disagreed on 7 percent of the reactors to either product. Similar analysis of the tests with 1:1000 H–15 injected in both arms shows there was disagreement between the tests in 6 percent of the persons with a reaction in either arm.

The agreement between the Lilly product and H–15 was even better. The two values for prevalence are 50.8 and 51.1 percent, respectively. The two products in the matching dilutions disagreed on the identity of only 2.2 percent of the reactors.

Although agreement on reactor frequencies might be considered the ultimate objective for matched products, it should be noted that histoplasmin reactor frequencies are relatively insensitive to potency changes.

Summary

Early in 1948 the supply of histoplasmin (H–15) employed by the Division of Tuberculosis in most of its studies of histoplasmin sensitivity was nearly exhausted. This paper describes the procedures used in developing a large new lot of histoplasmin to replace H–15.

Histoplasmin lots, like most biological preparations, are known to differ in potency. Since there was no standard reference product, it was decided to achieve continuity in the research programs by adjusting the potency of the new material to match the old. This was done by determining what dilution of the new material would match

the previously used 1:1000 dilution of H–15, arbitrarily called "the standard."

The estimation of the matching dilution followed the testing of three trial dilutions. Each trial dilution was compared to the standard by human skin testing in which the standard was placed in one arm and the trial dilution in the other. A sensitive measure of the relative potency of each trial dilution, called the critical difference, consisted of the difference of two percentages:

1. The percentage of persons whose reaction to the standard was larger;
2. The percentage of persons whose reaction to the trial dilution was the larger.

Critical differences for the three trial dilutions permitted estimation of a matching dilution associated with a zero critical difference, i. e., with equality of the two percentages.

Four lots of histoplasmin were considered in the selection of the new product. Tests of each lot and certain combinations of the lots led to the final selection of a pooled lot involving three of them. The estimated matching dilution for this pooled lot (H–42) was 8:1000.

Considerable use of a lot H–40 was made during the period of development of H–42. Comparison of this lot with H–15 indicates a matching dilution of approximately 3:1000. Estimates of the relative potency 1:1000 H–40 compared to the standard were made.

Tests of a lot of histoplasmin now being distributed by Eli Lilly Company revealed that a 1:100 dilution of this material (lot B–8287) was approximately equivalent to 1:1000 H–15.

Incidental to these assays, duplicate tests with the standard product were made to provide an estimate of the reproducibility of the histoplasmin skin test.

The analytical method employed (critical differences) appears to be fairly successful in achieving the objectives of the assay, namely, estimating the matching dilution for an unknown product.

Comparisons of the method with other techniques of assay are beyond the scope of this report, but it is planned to undertake such a comparison in an independent study.

ACKNOWLEDGMENT

This research was accomplished under the guidance of Dr. Carroll E. Palmer, Chief of Field Studies Branch, Division of Tuberculosis. The preparation of materials, the injections, and the readings were performed by the field staff of the Immunization Evaluation Section, Division of Tuberculosis, under the competent direction of Dr. Harold S. Barrett, Chief, Field Unit of this Section.

The opportunity for this research was created by the Departments of Health and Welfare of the State of Ohio. Particular thanks are due Dr. Arnold B. Kurlander, Chief of the Division of Tuberculosis, Department of Health, and Consultant in Internal Medicine, Department of Welfare of that State, and his staff for their cooperation and assistance in the execution of the field work and preliminary processing of the data.

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APPENDIX

Correlation between the degree of response [1] to the stated dilution of the test product and to the standard 1:1000 dilution of H–15

1:1000 H–36

	1:1000 H–15								
	A	B	C	D	E	F	G	H	Total
A	35	8	10	22	12	7	--	--	94
B	--	--	--	4	1	1	--	--	6
C	--	1	3	15	12	1	3	--	35
D	1	1	2	5	10	15	5	--	39
E	--	--	2	--	1	6	4	1	14
F	--	--	--	2	1	1	5	8	17
G	--	--	--	2	--	--	1	1	4
H	--	--	--	--	--	--	--	--	--
Total	36	10	17	50	37	31	18	10	209

1:1000 H–37

	1:1000 H–15								
	A	B	C	D	E	F	G	H	Total
A	41	2	5	23	41	23	5	1	141
B	--	--	--	--	1	--	--	--	1
C	1	--	--	2	5	9	6	--	23
D	1	--	--	2	10	17	21	4	55
E	--	--	--	--	1	7	16	3	27
F	--	--	--	1	2	--	5	2	10
G	--	--	--	--	--	--	1	--	1
H	--	--	--	--	--	--	--	--	--
Total	43	2	5	28	60	56	54	10	258

3:1000 H–36

	1:1000 H–15								
	A	B	C	D	E	F	G	H	Total
A	45	1	4	2	3	1	--	--	56
B	1	5	3	--	--	1	--	--	10
C	2	3	2	3	1	3	--	--	14
D	--	1	2	3	5	3	--	--	14
E	--	--	--	3	4	11	9	1	28
F	--	1	--	1	6	9	8	1	26
G	--	--	--	--	2	2	13	3	20
H	--	--	--	--	--	--	2	8	10
Total	48	11	11	12	21	30	32	13	178

5:1000 H–37

	1:1000 H–15								
	A	B	C	D	E	F	G	H	Total
A	53	--	9	8	4	2	3	--	79
B	--	--	--	--	--	--	--	--	--
C	--	--	2	8	4	3	--	1	18
D	--	--	--	10	12	12	4	--	38
E	--	--	--	4	4	2	7	--	17
F	--	--	--	--	2	2	1	1	6
G	--	--	--	--	--	1	1	2	4
H	--	--	--	--	--	--	--	--	
Total	53	--	11	30	26	22	16	4	162

10:1000 H–36

	1:1000 H–15								
	A	B	C	D	E	F	G	H	Total
A	38	--	2	--	--	--	--	--	40
B	3	2	1	2	1	--	--	--	9
C	3	1	4	5	2	1	--	--	16
D	2	3	11	22	9	2	--	--	49
E	1	2	1	12	8	3	1	--	28
F	--	--	1	6	13	4	3	--	27
G	--	--	--	2	--	5	5	--	12
H	--	--	--	--	--	1	1	--	2
Total	47	8	20	49	33	16	10	--	183

20:1000 H–37

	1:1000 H–15								
	A	B	C	D	E	F	G	H	Total
A	61	3	--	3	--	2	--	--	69
B	--	5	1	1	2	2	--	--	11
C	--	1	4	9	4	1	--	--	19
D	--	1	3	17	11	8	--	--	40
E	--	--	--	10	6	11	1	--	28
F	1	--	--	1	10	14	3	--	29
G	--	--	--	--	1	2	5	1	9
H	--	--	--	--	--	--	2	1	3
Total	62	10	8	41	34	40	11	2	208

1:1000 H–40

	1:1000 H–15								
	A	B	C	D	E	F	G	H	Total
A	70	2	--	2	1	1	--	--	76
B	--	--	--	2	--	--	--	--	2
C	--	--	--	--	2	1	--	--	3
D	1	--	--	11	25	4	1	--	42
E	--	--	--	9	17	25	6	1	58
F	--	--	--	--	6	2	6	2	16
G	--	--	--	--	1	--	6	2	9
H	--	--	--	--	--	--	--	--	--
Total	71	2	--	24	52	33	19	5	206

5:1000 H–40

	1:1000 H–15								
	A	B	C	D	E	F	G	H	Total
A	48	--	1	2	--	--	--	--	51
B	--	--	--	--	--	--	--	--	--
C	6	--	1	1	1	--	--	--	9
D	6	--	3	19	10	2	1	1	42
E	1	1	--	14	23	10	--	--	49
F	--	--	--	7	21	16	6	--	50
G	--	--	--	1	5	7	7	--	20
H	1	--	--	2	1	2	2	3	11
Total	62	1	5	46	61	37	16	4	232

[1] A = No response.
B = Erythema only, less than 10 mm.
C = Either: erythema only, 10 mm. or more
or: induration of 1–4 mm.
D = Induration of 5–7 mm.
E = Induration of 8–9 mm.
F = Induration of 10–11 mm.
G = Induration of 12–14 mm.
H = Induration of 15 mm. or more

APPENDIX—Continued

Correlation between the degree of response [1] to the stated dilution of the test product and to the standard 1:1000 dilution of H–15—Continued

1:1000 H–38

	A	B	C	D	E	F	G	H	Total
A	**163**	1	9	19	10	7	2	--	211
B	--	**1**	--	1	--	--	--	--	2
C	3	--	**7**	11	15	19	4	2	61
D	1	--	--	**6**	2	25	20	4	58
E	--	--	1	2	**2**	13	9	4	31
F	--	--	--	2	3	**1**	6	2	14
G	--	--	--	--	--	1	**1**	2	4
H	--	--	--	--	--	--	--	**5**	5
Total	167	2	17	41	32	66	42	19	386

1:1000 H–39

	A	B	C	D	E	F	G	H	Total
A	**29**	4	2	4	7	9	--	--	55
B	1	--	--	--	1	1	--	--	3
C	5	--	**4**	4	1	--	--	1	15
D	1	--	--	**5**	2	3	3	--	14
E	--	--	--	--	**3**	4	5	1	13
F	--	--	--	--	1	**1**	4	3	9
G	--	--	--	--	1	--	**2**	6	9
H	--	--	--	--	--	1	1	--	2
Total	36	4	6	13	16	19	15	11	120

1:1000 H–38

	A	B	C	D	E	F	G	H	Total
A	**138**	5	4	3	2	--	--	--	152
B	3	**2**	--	1	--	1	--	--	7
C	4	--	**1**	4	1	--	--	--	10
D	--	--	--	**1**	15	8	1	--	25
E	--	--	1	--	**7**	15	10	1	34
F	--	--	--	--	2	**5**	12	2	21
G	--	--	--	--	--	--	**9**	5	14
H	--	--	--	--	--	--	1	**11**	12
Total	145	7	6	9	27	29	33	19	275

1:1000 H–39

	A	B	C	D	E	F	G	H	Total
A	**44**	5	1	--	1	--	--	--	51
B	3	**11**	3	--	1	--	--	--	18
C	2	1	**2**	4	--	--	--	--	9
D	--	1	5	**17**	9	3	2	--	37
E	--	--	--	5	**4**	7	3	--	19
F	--	--	1	2	5	**7**	8	--	23
G	--	--	--	--	1	--	**10**	3	14
H	--	--	--	--	--	--	1	**1**	2
Total	49	18	12	28	21	17	24	4	173

1:1000 H–38

	A	B	C	D	E	F	G	H	Total
A	**156**	3	2	--	--	--	--	--	161
B	1	**4**	--	--	1	1	--	--	7
C	1	1	**4**	2	5	5	--	--	18
D	--	--	1	**7**	5	10	2	--	25
E	--	1	2	6	**7**	21	2	1	40
F	--	--	--	--	7	**31**	35	5	78
G	--	--	--	--	1	17	**14**	6	38
H	--	--	--	--	2	1	7	**48**	58
Total	158	9	9	15	28	86	60	60	425

1:1000 H–39

	A	B	C	D	E	F	G	H	Total
A	**28**	1	--	--	--	--	--	--	29
B	3	**5**	--	1	--	--	--	--	9
C	1	1	**7**	--	--	--	--	--	9
D	4	--	9	**8**	6	3	--	--	30
E	1	1	6	11	**5**	5	--	--	29
F	--	--	4	13	15	**6**	2	--	40
G	--	--	--	3	5	5	**5**	--	18
H	--	--	--	--	1	1	1	**1**	4
Total	37	8	26	36	32	20	8	1	168

1:1000 H–38

	A	B	C	D	E	F	G	H	Total
A	**22**	--	--	1	--	--	--	--	23
B	1	--	--	--	--	--	--	--	1
C	--	--	**2**	1	1	--	--	--	4
D	--	--	1	**7**	3	2	--	--	13
E	1	--	--	1	**3**	2	1	--	8
F	--	--	--	--	7	**8**	6	--	21
G	--	--	--	--	1	5	**5**	2	13
H	--	--	--	--	--	--	3	**4**	7
Total	24	--	3	10	15	17	15	6	90

1:1000 H–39

	A	B	C	D	E	F	G	H	Total
A	**48**	--	1	1	--	--	--	--	50
B	--	**3**	--	--	--	--	--	--	3
C	2	--	**13**	1	1	--	--	--	17
D	1	--	2	**27**	5	3	--	--	38
E	--	1	--	4	**22**	8	5	--	40
F	--	--	--	5	9	**35**	22	1	72
G	--	--	--	1	3	25	**15**	5	49
H	--	--	--	--	--	2	8	**10**	20
Total	51	4	16	39	40	73	50	16	289

[1] A = No response.
B = Erythema only, less than 10 mm.
C = Either: erythema only, 10 mm. or more
or: induration of 1–4 mm.
D = Induration of 5–7 mm.
E = Induration of 8–9 mm.
F = Induration of 10–11 mm.
G = Induration of 12–14 mm.
H = Induration of 15 mm. or more

Correlation between the degree of response [1] to the stated dilution of the test product and to the standard 1:1000 dilution of H–15—Continued

1:1000 H–15 (right arm)

1:1000 H–15 (left arm)

	A	B	C	D	E	F	G	H	Total
A	104	1	4	1	--	--	--	--	110
B	--	1	--	2	--	--	--	--	3
C	2	--	2	4	1	--	--	--	9
D	5	1	3	22	15	6	--	--	52
E	--	--	3	26	21	17	3	--	70
F	1	--	--	7	20	22	10	--	60
G	--	--	--	1	4	8	5	5	23
H	--	--	--	1	--	1	2	--	4
Total	112	3	12	64	61	54	20	5	331

1:1000 H–15

4:1000 H–41

	A	B	C	D	E	F	G	H	Total
A	114	1	3	2	--	4	--	--	124
B	--	--	--	1	--	1	--	--	2
C	--	1	2	6	3	2	--	1	15
D	--	--	3	13	8	11	3	--	38
E	1	--	2	1	5	42	5	--	56
F	1	--	1	--	9	56	49	6	122
G	--	--	--	--	2	29	20	4	55
H	--	--	--	--	--	--	5	1	6
Total	116	2	11	23	27	145	82	12	418

1:1000 H–15 (right arm)

1:1000 H–15 (left arm)

	A	B	C	D	E	F	G	H	Total
A	86	1	--	1	--	--	--	--	88
B	3	2	--	--	--	--	--	--	5
C	3	--	3	--	2	--	--	--	8
D	1	--	2	4	3	2	--	--	12
E	1	--	--	4	7	10	1	1	24
F	--	--	--	2	8	19	15	--	44
G	--	1	--	1	4	19	24	9	58
H	--	--	--	--	--	1	9	19	29
Total	94	4	5	12	24	51	49	29	268

1:1000 H–15

8:1000 H–41

	A	B	C	D	E	F	G	H	Total
A	115	--	2	1	1	1	--	--	120
B	2	--	1	--	1	--	--	--	4
C	--	--	--	3	3	--	--	--	6
D	--	1	2	14	14	13	1	1	46
E	1	--	1	14	8	14	10	--	48
F	--	1	--	8	31	42	23	2	107
G	--	--	--	1	6	22	20	11	60
H	--	--	--	--	1	2	9	16	28
Total	118	2	6	41	65	94	63	30	419

1:1000 H–15 (right arm)

1:1000 H–15 (left arm)

	A	B	C	D	E	F	G	H	Total
A	45	4	--	--	--	--	--	--	49
B	2	2	--	--	--	--	--	--	4
C	3	1	9	4	3	--	--	--	20
D	1	1	3	16	22	3	--	--	46
E	1	1	2	13	7	8	1	--	33
F	--	--	2	6	19	11	12	1	51
G	--	--	--	--	1	5	15	4	25
H	--	--	--	--	--	2	4	4	10
Total	52	9	16	39	52	29	32	9	238

1:1000 H–15

8:1000 H–41

	A	B	C	D	E	F	G	H	Total
A	79	1	1	1	--	--	--	--	82
B	1	1	--	--	1	--	--	--	3
C	1	--	2	3	1	2	--	--	9
D	--	--	4	2	7	7	--	1	21
E	--	--	1	3	5	18	--	1	28
F	3	2	--	--	5	21	22	2	55
G	--	--	--	--	4	21	25	4	54
H	--	--	--	--	1	4	5	6	16
Total	84	4	8	9	24	73	52	14	268

1:1000 H–15 (bottle No. 1)

1:1000 H–15 (bottle No. 2)

	A	B	C	D	E	F	G	H	Total
A	177	1	--	2	--	--	--	--	180
B	--	--	--	2	--	--	--	--	2
C	--	--	--	--	--	--	--	--	--
D	2	--	--	29	12	7	--	--	50
E	--	--	--	12	15	19	2	1	49
F	--	--	--	5	21	23	18	4	71
G	--	--	--	--	2	16	11	7	36
H	--	--	--	--	1	4	7	5	17
Total	179	1	--	50	51	69	38	17	405

1:1000 H–15

16:1000 H–41

	A	B	C	D	E	F	G	H	Total
A	42	--	--	1	--	--	--	--	43
B	2	3	1	--	--	--	--	--	6
C	4	1	2	--	--	--	--	--	7
D	3	1	7	9	6	3	--	--	29
E	--	1	1	10	9	2	2	--	25
F	--	--	1	6	13	10	6	--	36
G	--	1	--	2	6	9	8	3	29
H	--	--	--	--	--	3	5	5	13
Total	51	7	12	28	34	27	21	8	188

[1] A = No response.
B = Erythema only, less than 10 mm.
C = Either: erythema only, 10 mm. or more or: induration of 1–4 mm.
D = Induration of 5–7 mm.
E = Induration of 8–9 mm.
F = Induration of 10–11 mm.
G = Induration of 12–14 mm.
H = Induration of 15 mm. or more

APPENDIX—Continued

Correlation between the degree of response [1] to the stated dilution of the test product and to the standard 1:1000 dilution of H–15—Continued

4:1000 H–42 vs 1:1000 H–15

	A	B	C	D	E	F	G	H	Total
A	**131**	2	--	3	--	2	--	--	138
B	4	--	--	1	2	--	--	--	7
C	1	--	**3**	1	--	--	--	--	5
D	--	--	--	**3**	12	4	2	--	21
E	--	--	--	6	**7**	26	6	2	47
F	--	--	--	1	8	**31**	46	6	92
G	--	--	--	--	1	10	**56**	23	90
H	--	--	--	--	--	--	9	**19**	28
Total	136	2	3	15	30	73	119	50	428

10:1000 H–42 vs 1:1000 H–15

	A	B	C	D	E	F	G	H	Total
A	**58**	2	3	--	--	--	--	--	63
B	--	**1**	2	1	--	--	--	--	4
C	2	1	**12**	7	2	1	1	--	26
D	--	--	1	**3**	6	9	--	--	19
E	--	--	--	2	**8**	27	3	--	40
F	--	--	--	2	13	**22**	25	6	68
G	--	--	--	--	1	7	**6**	7	21
H	--	--	--	--	1	2	6	**22**	31
Total	60	4	18	15	31	68	41	35	272

8:1000 H–42 vs 1:1000 H–15

	A	B	C	D	E	F	G	H	Total
A	**116**	--	1	--	--	1	--	--	118
B	--	--	--	--	--	--	--	--	--
C	--	--	--	1	--	--	--	--	1
D	--	--	--	**3**	3	--	--	--	6
E	--	--	--	4	**7**	16	11	1	39
F	--	1	1	4	15	**35**	26	5	87
G	--	--	--	1	3	28	**102**	17	151
H	--	--	--	--	--	2	18	**28**	48
Total	116	1	2	13	28	82	157	51	450

10:1000 H–42 vs 1:1000 H–15

	A	B	C	D	E	F	G	H	Total
A	**141**	--	3	1	--	1	--	--	146
B	--	--	--	--	--	--	--	--	--
C	1	--	--	1	--	1	--	--	3
D	1	--	5	7	4	1	2	--	20
E	1	--	2	**1**	**3**	5	--	--	12
F	1	--	--	6	14	**25**	18	2	66
G	--	--	--	1	2	15	**17**	3	38
H	--	--	--	--	1	2	4	**18**	25
Total	145	--	10	17	24	50	41	23	310

8:1000 H–42 vs 1:1000 H–15

	A	B	C	D	E	F	G	H	Total
A	**69**	1	2	1	2	1	1	--	77
B	2	**5**	2	1	--	--	2	--	12
C	4	--	**6**	7	2	2	--	--	21
D	2	1	5	**1**	8	9	--	--	26
E	--	--	1	9	8	12	3	--	33
F	2	--	1	2	9	**21**	16	3	54
G	--	--	--	--	1	7	**12**	5	25
H	--	--	--	--	--	1	1	**4**	6
Total	79	7	17	21	30	53	35	12	254

10:1000 H–42 vs 1:1000 H–15

	A	B	C	D	E	F	G	H	Total
A	**132**	--	1	--	--	--	--	--	133
B	1	--	--	--	--	--	--	--	1
C	3	--	**4**	1	--	--	--	--	8
D	--	--	--	**11**	13	3	--	--	27
E	--	--	--	6	**9**	12	--	--	27
F	--	--	--	1	18	**40**	10	2	71
G	--	--	--	--	--	21	**14**	4	39
H	--	--	--	--	--	1	10	**4**	15
Total	136	--	5	19	40	77	34	10	321

16:1000 H–42 vs 1:1000 H–15

	A	B	C	D	E	F	G	H	Total
A	**50**	--	--	--	1	--	--	--	51
B	6	**1**	--	--	--	1	--	--	8
C	7	3	**3**	--	1	--	--	--	14
D	8	5	7	**4**	3	5	--	--	32
E	2	--	4	9	**2**	10	1	--	28
F	2	--	--	9	9	**9**	6	1	36
G	--	--	--	1	6	19	**14**	6	46
H	--	--	--	--	2	9	11	**14**	36
Total	75	9	14	23	24	53	32	21	251

10:1000 H–42 vs 1:1000 H–15

	A	B	C	D	E	F	G	H	Total
A	**327**	--	--	--	2	--	--	--	329
B	--	--	--	--	--	--	--	--	--
C	1	--	**1**	--	--	1	--	--	3
D	4	--	5	**18**	5	1	--	--	33
E	--	--	--	43	**18**	8	--	--	69
F	--	--	--	15	47	**35**	5	--	102
G	--	--	--	--	6	50	**5**	--	61
H	--	--	--	--	1	1	1	**1**	4
Total	332	--	6	76	79	96	11	1	601

[1] A = No response.
B = Erythema only, less than 10 mm.
C = Either: erythema only, 10 mm. or more; or: induration of 1–4 mm.
D = Induration of 5–7 mm.
E = Induration of 8–9 mm.
F = Induration of 10–11 mm.
G = Induration of 12–14 mm.
H = Induration of 15 mm. or more

APPENDIX—Continued

Correlation between the degree of response [1] to the stated dilution of the test product and to the standard 1:1000 dilution of H–15—Continued

4:1000 H–43 vs **1:1000 H–15**

	A	B	C	D	E	F	G	H	Total
A	140	..	..	1	1	1	..	..	143
B	..	..	..	..	..	..	..	..	..
C	..	..	1	..	5	3	..	..	9
D	..	..	..	2	4	3	..	..	9
E	..	..	..	2	3	5	3	..	13
F	..	..	..	..	5	3	9	8	25
G	..	..	..	..	..	4	4	10	18
H	..	..	..	..	..	1	..	6	7
Total	140	..	1	5	18	20	16	24	224

4:1000 H–44 vs **1:1000 H–15**

	A	B	C	D	E	F	G	H	Total
A	172	..	2	..	..	..	..	..	174
B	..	..	..	..	..	..	..	..	..
C	..	..	12	6	2	2	..	..	22
D	..	..	3	3	7	4	..	..	17
E	1	..	1	4	3	9	4	3	25
F	..	..	..	2	3	7	10	4	26
G	..	..	..	..	..	4	2	3	9
H	..	..	..	..	..	1	..	3	4
Total	173	..	18	15	15	27	16	13	277

8:1000 H–43 vs **1:1000 H–15**

	A	B	C	D	E	F	G	H	Total
A	325	1	..	..	..	..	..	..	326
B	..	2	..	1	..	..	..	..	3
C	4	..	14	4	1	1	..	..	24
D	..	..	2	13	8	4	..	..	27
E	..	..	1	12	5	17	4	..	39
F	..	..	..	5	14	16	16	8	59
G	..	..	..	..	3	12	10	2	27
H	..	..	..	..	2	3	4	16	25
Total	329	3	17	35	33	53	34	26	530

6:1000 H–44 vs **1:1000 H–15**

	A	B	C	D	E	F	G	H	Total
A	204	..	2	..	..	..	..	..	206
B	..	..	..	..	..	..	..	..	..
C	..	..	5	1	1	..	..	..	7
D	..	..	1	..	4	2	..	..	7
E	..	..	..	3	8	6	1	..	18
F	1	..	..	2	5	4	3	..	15
G	..	..	..	..	4	6	..	3	13
H	..	..	..	..	1	2	2	7	12
Total	205	..	8	6	23	20	6	10	278

1:1000 Lilly vs **1:1000 H–15**

	A	B	C	D	E	F	G	H	Total
A	106	..	1	4	..	3	1	..	115
B	..	..	..	..	..	..	..	..	..
C	1	..	1	7	5	9	3	3	29
D	..	..	1	..	3	16	7	15	42
E	..	..	..	..	..	4	3	12	19
F	..	..	..	1	..	3	2	6	12
G	..	..	..	..	..	1	1	4	6
H	..	..	..	..	..	..	..	1	1
Total	107	..	3	12	8	36	17	41	224

8:1000 H–44 vs **1:1000 H–15**

	A	B	C	D	E	F	G	H	Total
A	186	..	..	..	..	..	..	..	186
B	1	1	..	..	..	..	..	..	2
C	3	..	9	..	..	..	..	..	12
D	..	..	2	3	3	1	..	..	9
E	2	..	..	4	3	4	..	..	13
F	..	..	2	5	8	9	5	..	29
G	..	..	..	2	2	5	8	4	21
H	..	..	..	..	1	2	5	15	23
Total	192	1	13	14	17	21	18	19	295

1:100 Lilly vs **1:1000 H–15**

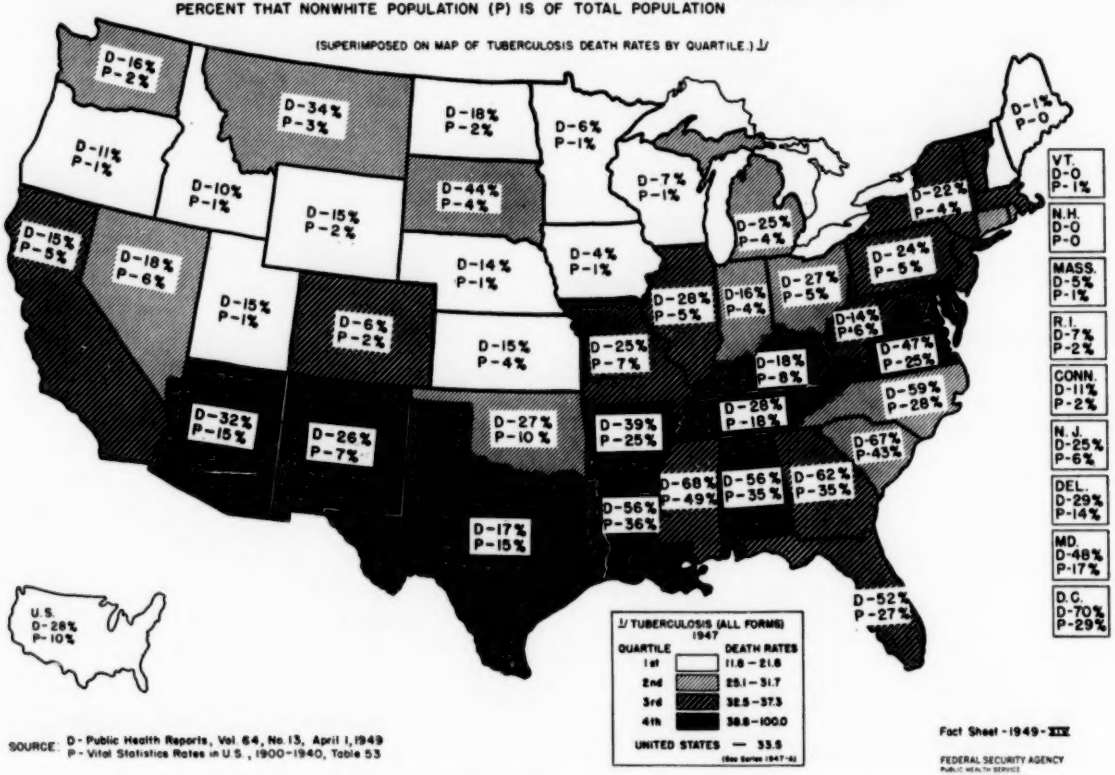
	A	B	C	D	E	F	G	H	Total
A	332	..	2	..	..	..	..	..	334
B	..	2	..	1	..	..	..	..	3
C	2	..	2	3	1	..	..	..	8
D	2	..	..	43	25	6	..	..	76
E	..	..	..	16	37	33	3	..	89
F	..	..	..	4	18	64	27	5	118
G	..	1	..	3	4	20	20	2	50
H	..	..	..	1	..	3	12	7	23
Total	336	3	4	71	85	126	62	14	701

[1] A = No response.
B = Erythema only, less than 10 mm.
C = Either: erythema only, 10 mm. or more
or: induration of 1–4 mm.
D = Induration of 5–7 mm.
E = Induration of 8–9 mm.
F = Induration of 10–11 mm.
G = Induration of 12–14 mm.
H = Induration of 15 mm. or more

PERCENT NONWHITE: TUBERCULOSIS DEATHS AND POPULATION BY STATES, 1947

PERCENT THAT NONWHITE TUBERCULOSIS DEATHS (D) ARE OF ALL TUBERCULOSIS DEATHS
PERCENT THAT NONWHITE POPULATION (P) IS OF TOTAL POPULATION

(SUPERIMPOSED ON MAP OF TUBERCULOSIS DEATH RATES BY QUARTILE.) 1/



1/ TUBERCULOSIS (ALL FORMS) 1947

QUARTILE	DEATH RATES
1st	11.8 – 21.8
2nd	25.1 – 31.7
3rd	32.5 – 37.3
4th	38.8 – 100.0

UNITED STATES — 33.9
(See Series 1947-4)

SOURCE: D - Public Health Reports, Vol. 64, No. 13, April 1, 1949
P - Vital Statistics Rates in U.S., 1900-1940, Table 53

Fact Sheet - 1949 - XIX

FEDERAL SECURITY AGENCY
PUBLIC HEALTH SERVICE
DIVISION OF TUBERCULOSIS

INCIDENCE OF DISEASE

No health department, State or local, can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

REPORTS FROM STATES FOR WEEK ENDED APRIL 15, 1950

Influenza

For the current week in the Nation, reported cases of influenza dropped sharply when compared with the preceding week, from 15,222 to 10,268. For the corresponding week last year 2,606 cases were reported. The cumulative total for the first 15 weeks of 1950 is 217,468 which may be compared with the corresponding total of 61,035 for the same period in 1949 and 266,137 for 1947, the highest on record during the past 5 years. The corresponding 5-year (1945–49) median is 126,054. Iowa reported 165 cases as compared with 1 last week.

Corrected figures from Kentucky give 224 cases of influenza for the current week and 331 for the preceding week.

Other Notifiable Diseases

Whooping cough increased from 2,373 cases reported last week to 2,467 for the current week which is above the 5-year (1945–49) median of 2,149. The cumulative total is 38,685 which is higher than the median of 32,906. The corresponding figure for 1949 was 14,971.

Infectious encephalitis increased from 8 cases last week to 24 for the current week. The 5-year median is 7 cases. The cumulative total for 15 weeks of 1950 is 199 which may be compared with the 5-year median of 116.

Diphtheria, measles, meningococcal meningitis, scarlet fever, typhoid fever, and Rocky Mountain spotted fever are all below their 5-year medians.

One case of anthrax was reported in Massachusetts; 62 cases of acute poliomyelitis were reported, 16 in California; and 16 cases of tularemia were reported for the current week. No smallpox was reported in the United States.

A typhoid epidemic among children aged 6 to 11 years in Spenard, a suburb of Anchorage, Alaska, was reported with 12 cases confirmed.

Telegraphic case reports from State health officers for the week ended April 15, 1950

[Leaders indicate that no cases were reported]

Division and State	Diphtheria	Encephalitis, infectious	Influenza	Measles	Meningitis, meningococcal	Pneumonia	Poliomyelitis	Rocky Mt. spotted fever	Scarlet fever	Smallpox	Tularemia	Typhoid and paratyphoid fever [1]	Whooping cough	Rabies in animals
NEW ENGLAND														
Maine			6	32		9			7				44	
New Hampshire			5			2							1	
Vermont	1			14	1				3				9	
Massachusetts	3	1		636	4				155			2	143	
Rhode Island			2	15		13			5				38	
Connecticut			10	69	1	60			36				121	
MIDDLE ATLANTIC														
New York	4	4	[2] 7	1,468	9	368	1		[3] 146			5	120	16
New Jersey		4	15	1,066	2	73			55			2	105	
Pennsylvania	6			565	7	161			113			4	151	
EAST NORTH CENTRAL														
Ohio	8		8	481	8	76	3		195			2	182	11
Indiana	9	3	20	445	1	10	1		49				48	
Illinois	1	3	31	517	5	108	2		62		2		74	2
Michigan	4	1	7	1,383	6	75	4		103				129	3
Wisconsin		1	159	487	3	12	1		86			3	128	
WEST NORTH CENTRAL														
Minnesota	3		35	147	1	36			23				60	
Iowa			165	970		4	1		7				4	8
Missouri			15	56	4	30			32		2		35	
North Dakota		2	78	16	1	21								
South Dakota	2			26		1			5				4	
Nebraska				108	1		2		9				1	
Kansas	1		5	49	3	21	1		21				34	
SOUTH ATLANTIC														
Delaware				4					1				5	
Maryland	1		36	48	2	46			22				49	
District of Columbia			1	67	2	8			2				1	
Virginia	4		1,448	108	1	109	2		15		2	1	76	4
West Virginia	3		658	321	2	13			8				59	11
North Carolina	6			177	1		1		14			1	43	
South Carolina	4		89	84	1	12			10			1	6	8
Georgia	2		101	88	1	17		1	10		3	2	8	11
Florida	2		7	129	1	21	1		3			1	5	1
EAST SOUTH CENTRAL														

Georgia	2		101	88	1	17		1	10			2	11	
Florida	2		7	129	1	21	1		3		1	5	1	
EAST SOUTH CENTRAL														
Kentucky	2		224	231	3	33			25			31	17	
Tennessee	2	1	175	165	4				26		1	20	14	
Alabama	7		420	187	3	78			11		1	19	10	
Mississippi	4	1	103	67	1	56			4	1	1	7	5	
WEST SOUTH CENTRAL														
Arkansas	1		2,145	47	4	87			7	4	2	32	3	
Louisiana	3		3	12		41	1		2			4		
Oklahoma	1		328	8	3	51			11		1	18	4	
Texas	17	1	3,531	731	5	505	14		36		5	237	20	
MOUNTAIN														
Montana			58	86	1	1			8	1		6		
Idaho			94	19					5			73		
Wyoming				26		2	1					1		
Colorado			18	59	1	26	6		6			6	2	
New Mexico				41		36	1		4		1	11		
Arizona			186	114		40	3		8			30	1	
Utah			2	264		3			2	1		25		
Nevada				7										
PACIFIC														
Washington	2		25	55	5	1			22			61		
Oregon			40	19	1	49			20		1	24		
California	4	2	8	534	5	33	16		119		2	179	1	
Total	107	24	10,268	12,248	104	2,348	62	1	1,513		16	39	2,467	153
Median, 1945–49	230	7	2,044	25,616	122		32	3	2,381	9	14	49	2,149	
Year to date 15 weeks	2,233	199	[4] 217,468	119,562	1,443	38,638	1,388	16	26,395	19	316	681	38,685	2,321
Median, 1945–49	4,231	116	126,054	250,733	1,322		573	10	41,671	77	294	683	32,906	
Seasonal low week ends	(27th) July 9		(30th) July 30	(35th) Sept. 3	(37th) Sept. 17		(11th) Mar. 18		(32d) Aug. 13	(35th) Sept. 3		(11th) Mar. 18	(39th) Oct. 1	
Since seasonal low week	6,504		[4] 247,998	138,692	2,356		251		42,834	39		171	60,221	
Median, 1944–45 to 1948–49	11,797		169,612	285,679	2,294		119		67,290	131		208	58,674	

[1] Including cases reported as salmonellosis.
[2] New York City only.
[3] Including cases reported as streptococcal sore throat.
[4] Deduction of 990 cases to agree with corrected report from Kentucky. Excludes 40,200 cases estimated by county health officers to have occurred in Kentucky during the period Jan. 1 to Apr. 8, 1950, and 4,000 cases estimated to have occurred in Jones County, Iowa.

Anthrax: Massachusetts, 1 case.
Alaska: Influenza 19, scarlet fever 7, typhoid 12.
Hawaii: No cases reported.

PLAGUE INFECTION IN THE STATE OF WASHINGTON

Under date of April 14, 1950, plague infection was reported proved in the following: A specimen of 378 fleas, 228 *Megabothris clantoni johnsoni*, 83 *Thrassis bacchi johnsoni*, 41 *Catallagia decipiens*, 25 *Monopsylla Wagneri* sp,. and 1 *Atyphloceras* sp., taken from 21 sagebrush voles, *Lagurus curtatus*, trapped March 29, 1950, 7 miles north of Farmer in Douglas County; and specimens of 420 and 520 fleas taken from 28 sagebrush voles, *Lagurus curtatus*, and 81 white-footed mice, *Peromyscus maniculatus*, respectively. The latter sagebrush voles and the white-footed mice were trapped April 7, 1950, 5 miles south of Wilber in Lincoln County.

TERRITORIES AND POSSESSIONS

Panama Canal Zone

Notifiable diseases—February 1950.—Certain notifiable diseases were reported in the Panama Canal Zone and terminal cities as follows:

Disease	Panama City		Colon		Canal Zone		Outside the zone and terminal cities		Total	
	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
Chickenpox	11				17		4		32	
Diphtheria				1			3		3	1
Dysentery:										
Amebic							7		7	
Bacillary	1				3		4		8	
Food poisoning, bacterial							1		1	
German measles					1				1	
Hepatitis, infectious							1		1	
Malaria [1]	1				4		66		71	
Measles	1		1		129		3		134	
Mumps	1				59				60	
Pneumonia		8		2	21	2			21	12
Tuberculosis		13		3	1	1			1	17
Typhoid fever							1		1	
Whooping cough	19		1	1	10		17		47	1

[1] 2 recurrent cases.

NOTE.—Cases are listed by place of residence except when place of infection is known.

DEATHS DURING WEEK ENDED APRIL 15, 1950

	Week ended Apr. 15, 1950	Corresponding week, 1949
Data for 94 large cities of the United States:		
Total deaths	9,718	9,232
Median for 3 prior years	9,232	
Total deaths, first 15 weeks of year	148,766	147,369
Deaths under 1 year of age	612	608
Median for 3 prior years	659	
Deaths under 1 year of age, first 15 weeks of year	9,458	9,915
Data from industrial insurance companies:		
Policies in force	69,822,892	70,481,914
Number of death claims	14,906	10,943
Death claims per 1,000 policies in force, annual rate	11.1	8.1
Death claims per 1,000 policies, first 15 weeks of year, annual rate	9.9	9.7

FOREIGN REPORTS

EGYPT

Cerebrospinal meningitis.—The high incidence of cerebrospinal meningitis continued to be reported in Cairo, Egypt, during the month of March 1950. Two hundred eighty-one cases were recorded in that city for the period March 4–25, with 33 deaths. In Alexandria 61 cases of the disease were reported during the period March 5–18.

FINLAND

Notifiable diseases—February 1950.—Cases of certain notifiable diseases were reported in Finland as follows:

Disease	Cases	Disease	Cases
Diphtheria	104	Scarlet fever	741
Dysentery	2	Typhoid fever	10
Meningitis, meningococcal	1	Venereal diseases:	
Paratyphoid fever	66	Gonorrhea	423
Poliomyelitis	8	Syphilis	36

JAPAN

Notifiable diseases—4 weeks ended February 25, 1950, and accumulated totals for the year to date.—Certain notifiable diseases were reported in Japan as follows:

Disease	4 weeks ended February 25, 1950		Total reported for the year to date	
	Cases	Deaths	Cases	Deaths
Diarrhea, infectious	10		10	
Diphtheria	1,277	130	2,458	261
Dysentery, unspecified	347	87	619	159
Filariasis	16		21	
Influenza	9,163		11,000	
Leprosy	31		58	
Malaria	51	4	88	11
Measles	4,107		7,308	
Meningitis, meningococcal	90	23	175	41
Paratyphoid fever	60	2	137	7
Pneumonia	19,957		39,064	
Poliomyelitis	114		248	
Puerperal infection	68		140	
Rabies	7		13	
Scarlet fever	319	3	692	5
Schistosomiasis	31		37	
Smallpox	1		3	1
Tetanus	91		205	
Trachoma	9,377		16,155	
Tuberculosis	31,392		56,842	
Typhoid fever	220	34	499	82
Typhus fever	476	27	495	29
Venereal diseases:				
Gonorrhea	12,973		24,119	
Syphilis	10,850		19,091	
Whooping cough	11,707		21,583	

NOTE.—The above figures include delayed and corrected reports.

MADAGASCAR

Notifiable diseases—February 1950.—Notifiable diseases were reported in Madagascar and Comoro Islands as follows:

Disease	Aliens		Natives	
	Cases	Deaths	Cases	Deaths
Beriberi			2	
Bilharziasis			48	1
Diphtheria	3		7	
Dysentery:				
Amebic	9	1	395	8
Bacillary			270	18
Erysipelas			24	
Influenza	23		3,462	72
Leprosy			50	
Malaria	327	3	43,589	282
Measles	1		58	
Meningitis, meningococcal			6	3
Mumps	2		195	
Paratyphoid fever	1			
Plague			9	6
Pneumonia:				
Broncho			273	69
Pneumococcic			328	65
Poliomyelitis			1	
Puerperal infection			2	
Scarlet fever	4		2	
Trachoma			1	
Tuberculosis, pulmonary	10	3	123	16
Typhoid fever	5		22	4
Whooping cough			264	14

REPORTS OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER RECEIVED DURING THE CURRENT WEEK

Note.—The following reports include only items of unusual incidence or of special interest and the occurrence of these diseases, except yellow fever, in localities which had not recently reported cases. All reports of yellow fever are published currently.

A table showing the accumulated figures for these diseases for the year to date is published in the PUBLIC HEALTH REPORTS for the last Friday in each month.

Cholera

India.—During the week ended April 1, 1950, 365 cases of cholera (with 136 deaths) were reported in Calcutta, and 346 cases were reported in that city for the week ended April 8.

Pakistan.—During the week ended April 1, 1950, three fatal cases of cholera were reported in Chittagong, and seven cases for the week ended April 8. In Dacca eight cases with six deaths were reported for the week ended March 25.

Plague

Burma.—Two fatal cases of plague were reported in the seaport of Henzada during the week ended March 11, 1950. During the week ended April 8, one case was reported in Rangoon.

India.—During the week ended April 8, 1950, plague was reported in certain cities in India as follows: Allahabad four cases (imported), Cawnpore two cases, Bombay one case.

Peru.—During the period February 1–28, 1950, three cases of plague were reported in Huancabamba Province, Piura Department.

Rhodesia (Northern).—During the week ended March 25, 1950, two fatal cases of plague were reported in Northern Rhodesia.

Venezuela.—During the period April 5–11, 1950, five cases of plague (one fatal) were reported in Tacata, Miranda State.

Smallpox

Bahrein Islands.—During the week ended April 1, 1950, 22 cases of smallpox were reported in Bahrein, Bahrein Islands.

Burma.—During the week ended March 25, 1950, 233 cases of smallpox, with 119 deaths, were reported in Burma, including 87 cases, 40 deaths, in Rangoon, and 33 cases, 17 deaths, in Bassein. One hundred seventy-two cases, with 98 deaths, were reported for the week ended April 1.

China.—During the period March 11–20, 1950, 31 cases of smallpox with 16 deaths were reported in Swatow. For the week ended April 1, 13 cases were reported in Shanghai.

India.—Smallpox has been reported in India as follows: Week ended April 1, 1950, Calcutta 242 cases, 205 deaths; Madras 174 cases, 38 deaths; week ended April 8, 1950, Calcutta 218 cases, Madras 178 cases.

Typhus Fever

Japan.—During the week ended April 1, 1950, 10 cases of typhus fever were reported in Tokyo and 19 cases in Yokohama.

Notice

A limited number of copies of "The Leading Causes of Death in the United States and States, 1947," issued this month by the Division of Tuberculosis, are available for free distribution. The 18-page pamphlet can be obtained upon request from the Publications Section, Division of Tuberculosis, Public Health Service, Washington, D. C.

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The printing of this publication has been approved by the Director of the Bureau of the Budget (August 10, 1949).

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It contains (1) current information regarding the incidence and geographic distribution of communicable diseases in the United States, insofar as data are obtainable, and of cholera, plague, smallpox, typhus fever, yellow fever, and other important communicable diseases throughout the world; (2) articles relating to the cause, prevention, and control of disease; (3) other pertinent information regarding sanitation and the conservation of the public health.

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